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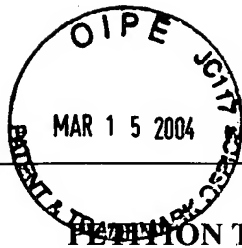
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**PETITION TO MAKE SPECIAL, REQUESTING THE ADVANCEMENT OF
EXAMINATION UNDER 37 C.F.R. § 1.102 AND M.P.E.P. § 708.02(II),**

First named inventor : Daly, John
App. No. : 10/658,093
Filed : September 9, 2003
Title : CONSTRUCTS FOR
GENE EXPRESSING
ANALYSIS
Examiner : Unknown

Group Art Unit: Unknown

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

March 10, 2004

(Date)

Jennifer A. Haynes, Ph. D., Reg. No. 46,868

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ATTN: Mail Stop DAC

Dear Sir:

Pursuant to 37 C.F.R. § 1.102, and M.P.E.P. § 708.02(II), Applicants hereby petition that the above application be made special due to actual infringement.

1. **A check in the amount of \$130 to cover the petition fee is enclosed herewith.**

Please charge any additional fees or credit overpayment to Deposit Account No. 11-1410.

2. **There is an infringing device or product actually on the market.**

The Applicant has discovered that Promega Corporation ("Promega") is selling vectors under the product name "Rapid Response™ Reporter Vectors", which fall within the scope of the pending claims. From the information available on their website (<http://www.promega.com/newprod/>), Promega lists this product under the subheading 'PROTEOMICS'. Activation of the hyperlink associated with the product name "Rapid Response™ Reporter Vectors" redirects the viewer to a webpage that describes these vectors in more detail, and that provides a price list and conditions for sale of those vectors. A **Technical Manual (#TM242)[Appendix A]** is listed on this webpage whose hyperlink redirects the viewer to an electronic copy of this manual. Hard copies of the web pages and technical manual are enclosed as Appendix A.

3. **A rigid comparison of this alleged infringing device or products with the claims has been made. In my opinion some of the claims are unquestionably infringed.**

The enclosed technical manual shows that vectors pGL3 (R2.2)-basic and phRG(R2.2)-basic each comprise a reporter gene operably linked to nucleic acid sequences encoding a protein degradation sequence and an mRNA degradation sequence (see page 3 of the Manual). The inventors have informed us that the intracellular half-life of the reporter protein expressed from these vectors is less than 1 hour. Accordingly, it is our opinion that these vectors fall within the scope of several of the claims.

4. A careful and thorough search of the prior art has been made.

A pre-examination search has been made of the presently claimed invention by the Australian Intellectual Property Office acting as the International Searching Authority for the international application of which the present application is a continuation-in-part. The results of this search are reported in the International Search Report for this international application. A copy of the **International Search Report [Appendix B]** and the documents cited are enclosed in the accompanying Information Disclosure Statement.

5. An Information Disclosure Statement and PTO form 1449 with 9 references enclosed.

6. A return prepaid postcard is enclosed.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: March 10, 2004

By: 

Jennifer A. Haynes, Ph.D.

Registration No. 48,868

Agent of Record

Customer No. 20,995

(415) 954-4114

Rapid Response™ Reporter Vectors



Technical Manual No. 242

INSTRUCTIONS FOR USE OF PRODUCTS E6431, E6441, E6451 AND E6461
All technical literature is available on the Internet at www.promega.com
Please visit the web site to verify that you are using the most current version of this Technical Manual.

I. Description	1
II. Product Components	2
III. General Considerations	2
A. Characteristics of the Rapid Response™ Luciferase Genes.....	2
B. Characteristics of the Rapid Response™ Luciferase Proteins.....	3
C. Protein Degradation Rate and Signal Intensity.....	5
IV. Rapid Response™ Reporter Vector Maps	7
V. References	11
VI. Related Products	11
VII. Appendix	13
A. pGL3(R2.1)-Basic Vector Restriction Enzyme Tables.....	13
B. pGL3(R2.2)-Basic Vector Restriction Enzyme Tables.....	15
C. phRG(R2.1)-Basic Vector Restriction Enzyme Tables.....	17
D. phRG(R2.2)-Basic Vector Restriction Enzyme Tables.....	19

I. Description

Firefly and *Renilla* luciferases are widely used to monitor transcriptional activity in cell biology and drug discovery applications because they are highly sensitive, flexible and easily quantified. However, these reporter proteins are relatively stable (Table 1) in mammalian cells and thus typically require several hours of incubation in order to detect changes in transcriptional activities. To reduce incubation times and shorten the time between transcription and reporter detection, Promega has designed luciferase genes that encode reporter proteins with reduced half-lives. The proteins encoded by these Rapid Response™ luciferase genes respond more quickly and with greater magnitude to changes in transcriptional activity than their more stable predecessors.

This Technical Manual provides information on the use of four Rapid Response™ Reporter Vectors(a,b,c)—the pGL3(R2.1)-Basic(a,b,c,d,e), pGL3(R2.2)-Basic(a,b,c,d,e), phRG(R2.1)-Basic(a,b,c,f) and phRG(R2.2)-Basic(a,b,c,f) Vectors. The pGL3(R2.1) and pGL3(R2.2) Vectors encode derivatives of firefly luciferase, and the phRG(R2.1) and phRG(R2.2) Vectors encode derivatives of *Renilla* luciferase. The R2.1 vectors (pGL3(R2.1)-Basic and phRG(R2.1)-Basic) encode reporter proteins that have reduced half-lives. The R2.2 vectors (pGL3(R2.2)-Basic and phRG(R2.2)-Basic) possess elements that reduce mRNA and protein half-lives.



A promoter or response element is required for reporter gene expression, thus one pertinent to your studies must be cloned into the Rapid Response™ Vectors.

Usage Information

The Rapid Response™ Reporter Vector backbone is Promega's pGL3-Basic(d,g) Vector, which does not contain promoter elements. A promoter or response element is required for reporter gene expression, thus one pertinent to your studies must be cloned into the Rapid Response™ Vectors. Use of the Rapid Response™ Reporter Vectors with Promega's firefly and *Renilla* Luciferase Assay Systems and reagents requires optimization.

II. Product Components

Product	Size	Cat.#
pGL3(R2.1)-Basic Vector(a,b,c,d,e)	20µg	E6431
pGL3(R2.2)-Basic Vector(a,b,c,d,e)	20µg	E6441
phRG(R2.1)-Basic Vector(a,b,c,f)	20µg	E6451
phRG(R2.2)-Basic Vector(a,b,c,f)	20µg	E6461

Storage Conditions: Store the Rapid Response™ Reporter Vectors at -20°C.

III. General Considerations

A. Characteristics of the Rapid Response™ Luciferase Genes

Firefly and *Renilla* luciferases are monomeric proteins that do not require post-translational modification for activity. Therefore, these enzymes can function as genetic reporters immediately following translation. Computational models predict that genetic reporters with reduced intracellular stability will yield faster response to changes in transcriptional rate and an increase in the relative magnitude of the response (1). Destabilized reporter proteins (i.e., those with faster protein degradation rates) are therefore expected to be more responsive and better suited to monitor rapid processes (such as promoter activation and repression) than reporters with slower protein degradation rates.

Transcription Factor Binding Sites Removed and Codon Usage Optimized

Native sequences for firefly and *Renilla* luciferase contain multiple consensus sequences for mammalian transcription factor binding sites. To increase the expression and reliability of Rapid Response™ Reporter Vectors, the luciferase genes present in these vectors were systematically redesigned, removing most of the consensus sequences for mammalian transcription factor binding sites. Additionally, the codons in both firefly and *Renilla* luciferase reporter genes were optimized for use in mammalian cells, creating the synthetic *hluc+* and *hRluc* genes, respectively.

Degradation Sequences Added

To generate reporter proteins that have increased protein degradation rates (i.e., destabilized reporters) two different degradation sequences have been incorporated into the synthetic firefly and *Renilla* luciferase genes. The first degradation sequence, PEST, is a forty-amino acid sequence isolated from the C-terminal region of mouse ornithine decarboxylase (2). The second consists of CL1, PEST and one mRNA degradation sequence, ARE (AU-rich element). CL1 has been shown to increase protein degradation (3). PEST and CL1-PEST have been designed to reduce the number of mammalian consensus transcription factor binding sites. ARE is a synthetic consensus sequence based on the 3'-untranslated region of *Herpesvirus saimiri* small nuclear RNA. These ARE are present in the 3'-untranslated regions of many early response genes where their presence has been shown to promote mRNA degradation.

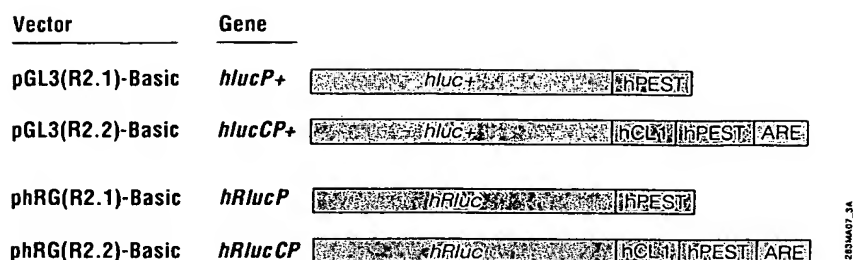


Figure 1. Diagram of the Rapid Response™ Reporter Genes.

The resulting synthetic versions of these degradation sequences are referred to as hPEST and hCL1-hPEST-ARE. The pGL3(R2.1)-Basic and the phRG(R2.1)-Basic Vectors contain the *hlucP+* (firefly) and *hRlucP* (*Renilla*) luciferase genes, respectively, both of which contain the hPEST degradation sequence. The pGL3(R2.2)-Basic and the phRG(R2.2)-Basic Vectors contain the *hlucCP+* (firefly) and the *hRlucCP* (*Renilla*) genes, respectively, and both genes contain the hCL1-hPEST-ARE degradation sequence (Figure 1). The vector backbone used for the four Rapid Response™ Vectors is the pGL3-Basic Vector (Cat.# E1751).

B. Characteristics of the Rapid Response™ Luciferase Proteins

Table 1 shows the reduction in reporter half-life of the luciferase proteins expressed from the Rapid Response™ Vectors. The half-lives of the firefly and *Renilla* Rapid Response™ Vectors have been reduced by more than 60% when compared to the parent synthetic firefly and *Renilla* luciferase proteins.

Because of this reduction in half-life, there is a corresponding reduction in time to achieve maximum-fold induction for all of the Rapid Response™ Reporter proteins (Table 1 and Figure 2). For firefly luciferase, the time to reach maximum fold induction for the control synthetic firefly luciferase gene (*hluc+*) is 6 hours. The firefly-based Rapid Response™ Reporters, *hlucP+* and *hlucCP+*, reach maximum-fold induction in 3.0 and 1.5 hours, respectively. For the *Renilla*-based Rapid Response™ constructs, *hRlucP* and *hRlucCP+*, the time to achieve maximum-fold induction compared to the control protein is also reduced. The *hRluc* control reaches maximum-fold induction at 8 hours, while the *hRlucCP* and *hRlucP* reach maximum-fold induction at 3.0 and 4.5 hours, respectively.



Note: Varying assay conditions will affect assay results.

Table 1. Characteristics of the Rapid Response™ Luciferase Reporter Proteins.

Vector	pGL3-Control	pGL3-hluc+ ²	pGL3 (R2.1)-Basic	pGL3 (R2.2)-Basic	phRG-B	phRG (R2.1)-Basic	phRG (R2.2)-Basic
Expressed Reporter Protein	luc+	hluc+	hlucP+	hlucCP+	hRluc	hRlucP	hRlucCP
Reporter Protein Half-Life (hours) ¹	3.4	3.2	1.0	0.6	3.0	1.0	0.4
Time to Achieve Maximum Induction (hours)	6.0	6.0	3.0	1.5	8.0	4.5	3.0

¹Rapid Response™ Reporters and control synthetic luciferase genes were cloned into the pGL3-Control Vector(d,g) and transfected into CHO cells. Twenty-four hours later, cycloheximide (100µg/ml final concentration) was added to each well. At specific time points, cells were collected and the relative light units determined using either the Luciferase Assay System(b,h) or the *Renilla* Luciferase Assay System(a,b). The firefly luc+ and hluc+ reporter proteins are 99.8% identical, with only one amino acid difference.

²pGL3-hluc+ is not commercially available.

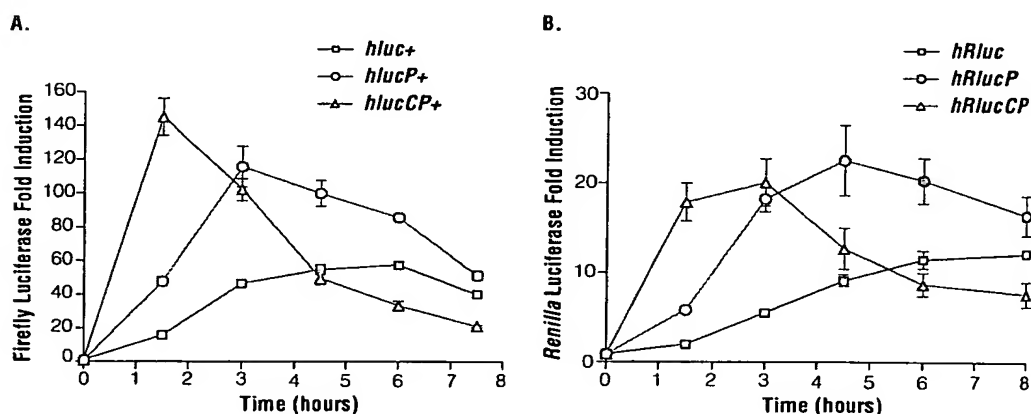


Figure 2. Time to achieve maximum-fold induction. To determine maximum-fold induction a DNA segment containing multiple CREs (cAMP Response Element) was cloned into each of the four Rapid Response™ Reporter Vectors and the two control vectors containing *hluc+* and *hRluc*. The CRE-containing vectors were then transiently transfected into d293 cells (a subpopulation of HEK 293 cells). Twenty-four hours later 100µM of RO (RO-20-1724) and 1µM of ISO (isoproterenol hydrochloride) were added to the transiently-transfected cells to induce reporter gene expression. RO alone (100µM) was added to a subset of the wells to serve as a noninduced control. Cells were harvested, lysed and assayed with either the Luciferase Assay System (Cat.# E1500; **Panel A**) or the *Renilla* Luciferase Assay System (Cat.# E2810; **Panel B**). Fold induction was calculated by dividing the relative light units obtained from the induced wells by the relative light units obtained from noninduced wells. Due to a large genetic difference between *hluc+* and *luc+* (>30%), *hluc+* was selected as the appropriate control.

The data in Figure 2 further detail the responsiveness of the Rapid Response™ Reporter Vectors. In Panel A at 1.5 hours the hluc+ control reaches ~16-fold induction. However, hlucCP+ and hlucP+ reach 145- and 48-fold induction, respectively, in the same 1.5 hours. Thus the percentage increase in reporter response (see equation below) for hlucCP+ and hlucP+ has increased 806% and 200%, respectively. In Panel B at 1.5 hours the hRluc control displays a 2-fold increase while hRlucCP displays a 18-fold increase over the same time frame; thus hRlucCP displays an 800% increase in reporter response. For the hRlucP a 6-fold increase is demonstrated for the 1.5 hour time point or a 200% increase in reporter response is demonstrated.

Reporter response is the percent change in the fold induction for a Rapid Response™ Reporter Vector over its respective control for the same time interval.

Reporter response =

$$\frac{[(\text{fold induction for hlucCP+}) - (\text{fold induction for hluc+})]}{(\text{fold induction for hluc+})} \times 100$$

$$\text{Reporter response for hlucCP+} = \frac{[145 - 16]}{16} \times 100 = 806\%$$

C. Protein Degradation Rate and Signal Intensity

Destabilized luciferase proteins are not accumulated in the cell to the same extent as their wildtype counterparts. As a result, destabilized reporter proteins typically generate lower signal intensities. The hlucP+ protein displays 51% (in CHO cells) and 203% (in NIH3T3 cells) of the relative light units obtained using the firefly luciferase gene (*luc+*) (Figure 3). Firefly luciferase hlucCP+ protein displays approximately 2% (in both CHO and NIH3T3 cells) of the relative light units of the pGL3-Control firefly *luc+* control (Figure 3, Panel A).

In Figure 3, Panel B, the reporter protein hRlucP displays 18.6% (in CHO cells) and 3.2% (in NIH3T3 cells) of the relative light units of the hRluc control. As with the hlucCP+ protein, the *Renilla* hRlucCP reporter protein displays the lowest signal intensity, having 10.3% (in CHO cells) and 0.6% (in NIH3T3 cells) of the relative light units of the hRluc control. Note that the relative light units obtained using the Rapid Response™ Reporter Vectors can vary depending on the mammalian cell line used.



Relative light units obtained using the Rapid Response™ Reporters can vary depending on the mammalian cell line used.

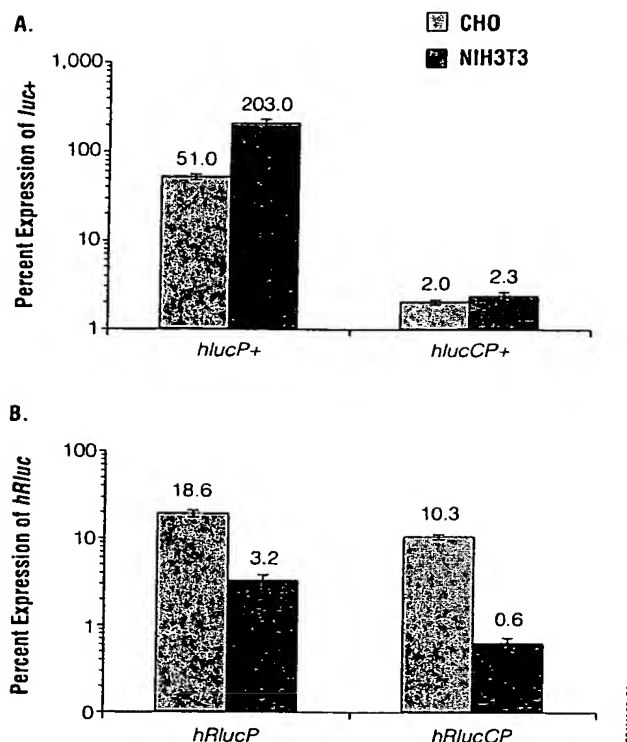


Figure 3. Expression of Rapid Response™ luciferase genes in CHO and NIH3T3 cells. The *luc+* gene present in pGL3-Control Vector (Cat.# E1741) was replaced with genes encoding either the *hLucP+*, *hLucCP+*, *hRluc*, *hRlucP*, or *hRlucCP* proteins. Thus the only difference among the various vectors is the reporter gene. The resulting vectors and the pGL3-Control Vector were cotransfected with a second reporter (transfection control) into either CHO or NIH3T3 cells. The transfection controls for **Panels A and B** were phRL-TK (Cat.# E6291) and pGL3-Control (Cat.# E1741) Vectors, respectively. Twenty-four hours post-transfection the cells were harvested with Passive Lysis Buffer (Cat.# E1941), and relative light units were determined using the Dual-Luciferase® Assay System^(d,h,i) (Cat.# E1910). The relative light units were normalized to the transfection control. The effects of the destabilization sequences on the accumulation of luciferase enzyme are shown as percent of the control. **Panel A.** Percent expression of *hLucP+* and *hLucCP+* versus *luc+* (pGL3-Control Vector) in CHO and NIH3T3 cell transfections. **Panel B.** Percent expression of *hRlucP* and *hRlucCP* versus *hRluc* in CHO and NIH3T3 cell transfections.

IV. Rapid Response™ Reporter Vector Maps

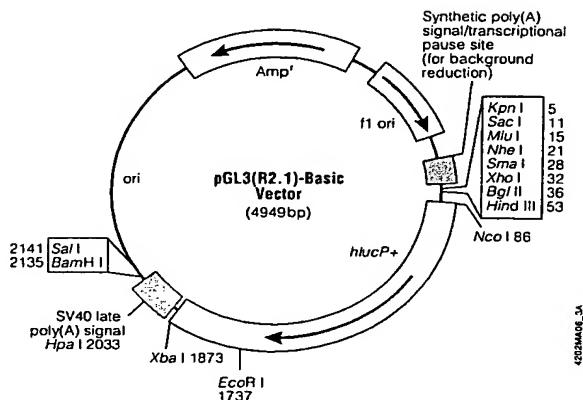


Figure 4. pGL3(R2.1)-Basic Vector circle map.

pGL3(R2.1)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
<i>hucP+</i> reporter gene (synthetic firefly luciferase; includes hPEST)	88–1863
SV40 late poly(A) region	1903–2124
Reporter Vector Primer 4 (RVPrimer4) binding site	2192–2211
<i>ColE1</i> -derived origin of replication	2449
β -lactamase (<i>Amp^r</i>) coding region	3211–4071
<i>f1</i> origin of replication*	4203–4658
Upstream synthetic poly(A) region	4789–4942
Reporter Vector Primer 3 (RVPrimer3) binding site	4891–4910

**f1* origin of replication verified by sequence only

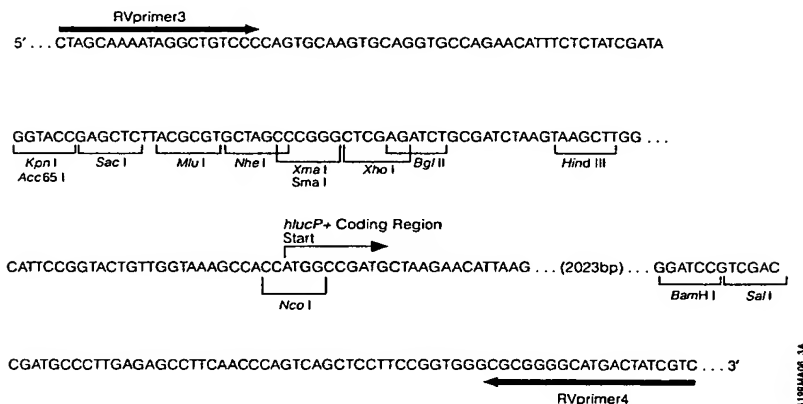


Figure 5. pGL3(R2.1)-Basic Vector synthetic firefly luciferase flanking regions including the multiple cloning region.

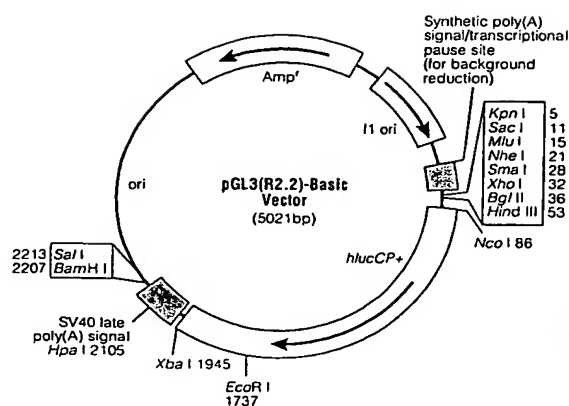


Figure 6. pGL3(R2.2)-Basic Vector circle map.

pGL3(R2.2)-Basic Vector Sequence Reference Points:

Multiple cloning region	1-58
<i>hLucCP+</i> reporter gene (synthetic firefly luciferase; includes hCL1, hPEST and ARE)	88-1944
SV40 late poly(A) region	1975-2196
Reporter Vector Primer 4 (RVPrimer4) binding site	2264-2283
<i>ColE1</i> -derived origin of replication	2521
β -lactamase (<i>Amp^r</i>) coding region	3283-4143
<i>f1</i> origin of replication*	4275-4730
Upstream synthetic poly(A) region	4861-5014
Reporter Vector Primer 3 (RVPrimer3) binding site	4963-4982

**f1* origin of replication verified by sequence only

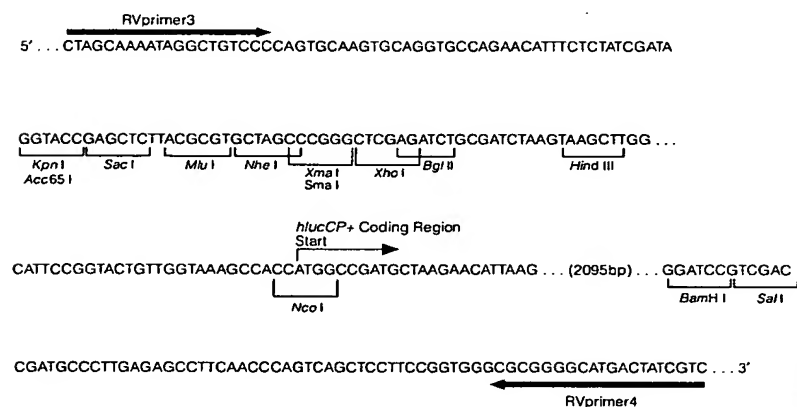


Figure 7. pGL3(R2.2)-Basic Vector synthetic firefly luciferase flanking regions including the multiple cloning region.

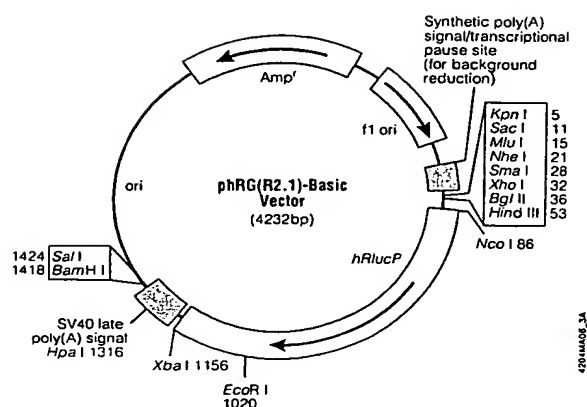


Figure 8. phRG(R2.1)-Basic Vector circle map.

phRG(R2.1)-Basic Vector Sequence Reference Points:

Multiple cloning region	1–58
<i>hRlucP</i> reporter gene (synthetic <i>Renilla</i> luciferase; includes hPEST)	88–1146
SV40 late poly(A) region	1186–1407
Reporter Vector Primer 4 (RVPrimer4) binding site	1475–1494
<i>ColE1</i> -derived origin of replication	1732
β -lactamase (<i>Amp^r</i>) coding region	2494–3354
<i>f1</i> origin of replication*	3486–3941
Upstream synthetic poly(A) region	4072–4225
Reporter Vector Primer 3 (RVPrimer3) binding site	4174–4193

**f1* origin of replication verified by sequence only

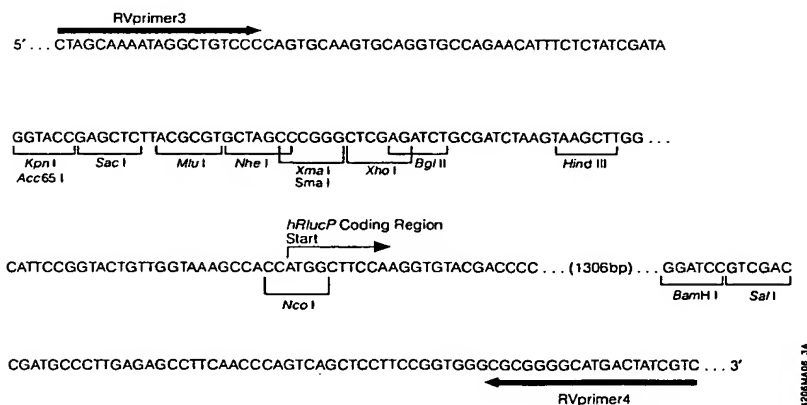


Figure 9. phRG(R2.1)-Basic Vector synthetic *Renilla* luciferase flanking regions including the multiple cloning region.

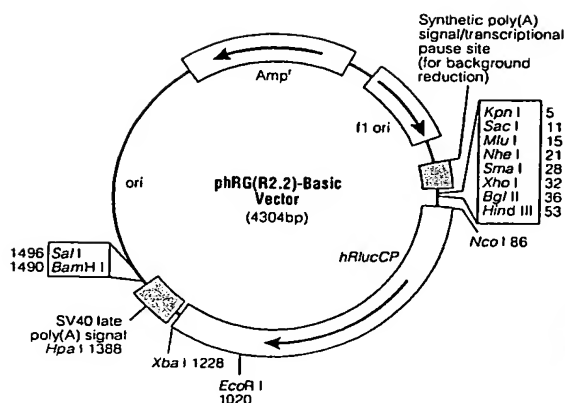


Figure 10. phRG(R2.2)-Basic Vector circle map.

phRG(R2.2)-Basic Vector Sequence Reference Points:

Multiple cloning region	1-58
<i>hRlucCP</i> reporter gene (synthetic <i>Renilla</i> luciferase, including hCL1, hPEST and ARE)	88-1227
SV40 late poly(A) region	1258-1479
Reporter Vector Primer 4 (RVPrimer4) binding site	1547-1566
<i>ColE1</i> -derived origin of replication	1804
β -lactamase (<i>Amp^r</i>) coding region	2566-3426
<i>f1</i> origin of replication*	3558-4013
Upstream synthetic poly(A) region	4144-4297
Reporter Vector Primer 3 (RVPrimer3) binding site	4246-4265

**f1* origin of replication verified by sequence only

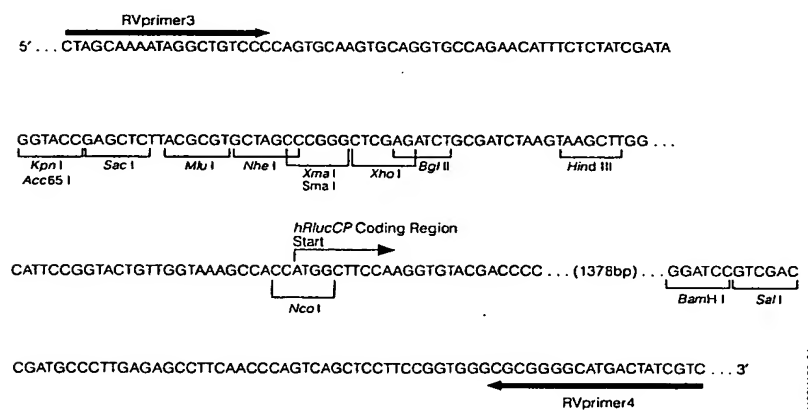


Figure 11. phRG(R2.2)-Basic Vector synthetic *Renilla* luciferase flanking regions including the multiple cloning region.

V. References

1. Schimke R.T. (1973) Control of enzyme levels in mammalian tissues. *Adv. Enzymol. Related Areas Mol. Biol.* **37**, 135–87.
2. Li, X. *et al.* (1998) Generation of destabilized green fluorescent protein as a transcription reporter. *J. Biol. Chem.* **273**, 34970–5.
3. Gilon, T., Chomsky, O. and Kulka, R.G. (1998) Degradation signals for ubiquitin system proteolysis in *Saccharomyces cerevisiae*. *EMBO. J.* **17**, 2759–66.
4. Fan, X.C., Myer, V.E. and Steitz, J.A. (1997) AU-rich elements target small nuclear RNAs as well as mRNAs for rapid degradation. *Genes Dev.* **11**, 2557–68.
5. Lagnado, C.A., Brown, C.Y. and Goodall, G.J. (1994) AUUUA is not sufficient to promote poly(A) shortening and degradation of an mRNA: The functional sequence within AU-rich elements may be UUAUUUA (U/A)(U/A). *Mol. Cell. Biol.* **14**, 7984–95.
6. Zubiaga, A.M., Belagco, J.G. and Greenberg, M.E. (1995) The nonamer UUAUU-UUUU is the key AU-rich sequence motif that mediates mRNA degradation. *Mol. Cell. Biol.* **15**, 2219–30.

VI. Related Products

Firefly and *Renilla* Luciferase Assay Systems

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System ^(b,i,j)	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Bright-Glo™ Luciferase Assay System ^(b,i,j)	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Steady-Glo® Luciferase Assay System ^(b,i,j)	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Luciferase Assay System ^(b,h)	100 assays	E1500
Luciferase 1000 Assay System ^(b,h)	1,000 assays	E4550
Luciferase Assay System with Reporter Lysis Buffer ^(b,h)	100 assays	E4030
Dual-Luciferase® Reporter Assay System ^(b,i,j)	100 assays	E1910
Dual-Luciferase® Reporter 1,000 Assay System ^(b,i,j)	1,000 assays	E1980
<i>Renilla</i> Luciferase Assay System ^(a,b)	100 assays	E2810
	1,000 assays	E2820

Lysis Buffer

Product	Size	Cat.#
Passive Lysis 5X Buffer	30ml	E1941



VI. Related Products (continued)

Synthetic *Renilla* Luciferase Vectors

Product	Size	Cat.#
phRL-null Vector(a,b,c,h)	20µg	E6231
phRL-TK Vector(a,b,c,h)	20µg	E6241
phRL-TK(Int-) Vector(a,b,c,h)	20µg	E6251
phRL-SV40 Vector(a,b,c,h)	20µg	E6261
phRL-CMV Vector(a,b,c,h,k)	20µg	E6271
phRG-B Vector(a,b,c,h)	20µg	E6281
phRG-TK Vector(a,b,c,h)	20µg	E6291

Firefly Luciferase Vectors

Product	Size	Cat.#
pGL3-Control Vector(d,g)	20µg	E1741
pGL3-Basic Vector(d,g)	20µg	E1751
pGL3-Promoter Vector(d,g)	20µg	E1761
pGL3-Enhancer Vector(d,g)	20µg	E1771

VII. Appendix

A. pGL3(R2.1)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3'-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487821) and online at: www.promega.com/vectors/

Table 2. Restriction Enzymes That Cut the pGL3(R2.1)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Acc I	2	688, 2142	EcoCR I	1	9
Acc65 I	1	1	EcoN I	3	277, 700, 1705
Afl III	2	15, 2391	EcoR I	1	1737
Alw26 I	4	1111, 1409, 3345, 4121	Ehe I	3	369, 1035, 1167
Alw44 I	2	2705, 3951	Fse I	2	1499, 1892
AlwN I	1	2807	Fsp I	3	910, 3506, 4679
Apa I	1	118	Hinc II	2	2033, 2143
Ava I	3	26, 32, 1756	Hind II	2	2033, 2143
Ava II	4	1209, 1392, 3422, 3644	Hind III	1	53
Bal I	2	1069, 1594	Hpa I	1	2033
BamH I	1	2135	Kas I	3	367, 1033, 1165
Ban II	4	11, 33, 118, 4362	Kpn I	1	5
Bbe I	3	371, 1037, 1169	Mlu I	1	15
Bbs I	1	2220	MspA1 I	5	151, 162, 2733, 2978, 3919
Bcl I	3	668, 1409, 1703	Nar I	3	368, 1034, 1166
Bgl I	5	88, 1492, 1667, 3404, 4672	Nci I	5	27, 28, 2771, 3467, 3818
Bgl II	1	36	Nco I	1	86
Bsa I	1	3345	Nhe I	1	21
BsaA I	2	1290, 4433	Not I	1	4782
BsaB I	3	535, 1702, 2134	Nsp I	3	579, 834, 2395
BsaM I	3	60, 1954, 2047	PaeR7 I	1	32
Bsm I	3	60, 1954, 2047	PshA I	1	2206
Bsp120 I	1	114	PspA I	1	26
BspH I	3	671, 3111, 4119	Pst I	3	347, 488, 1466
BspM I	2	1019, 4912	Pvu I	2	3654, 4700
BsrG I	1	578	Pvu II	1	162
BssS I	3	1015, 2564, 3948	Sac I	1	11
BstX I	1	1206	Sal I	1	2141
BstZ I	3	1886, 1890, 4782	Sca I	3	253, 3764, 4847
Bsu36 I	2	701, 1301	Sin I	4	1209, 1392, 3422, 3644
Cla I	3	2128, 4840, 4944	Sma I	1	28
Dra I	4	2094, 3150, 3169, 3861	Srf I	1	28
Dra II	4	114, 115, 480, 1659	Ssp I	3	4088, 4641, 4756
Dra III	1	4436	Sty I	3	86, 1199, 1655
Drd I	3	1876, 2499, 4480	Tfi I	2	2366, 4837
Eag I	3	1886, 1890, 4782	Vsp I	1	3456
Ear I	5	172, 283, 2275, 4079, 4717	Xba I	1	1873
EcHK I	1	3284	Xho I	1	32
Eco47 III	2	1075, 2267	Xma I	1	26
Eco52 I	3	1886, 1890, 4782	Xmn I	1	3883
Eco81 I	2	701, 1301			

Note: The enzymes listed in boldface type are available from Promega.



Table 3. Restriction Enzymes That Do Not Cut the pGL3(R2.1)-Basic Vector.

<i>Aat</i> II	<i>Bbu</i> I	<i>Csp45</i> I	<i>Pfl</i> M I	<i>Sac</i> II	<i>Sse8387</i> I
<i>AccB7</i> I	<i>Blp</i> I	<i>Eco72</i> I	<i>PinA</i> I	<i>Sfi</i> I	<i>Stu</i> I
<i>Acc</i> III	<i>Bpu</i> 1102 I	<i>EcoR V</i>	<i>Pme</i> I	<i>Sgf</i> I(l)	<i>Swa</i> I
<i>Afl</i> II	<i>BssH</i> II	<i>I-Ppo</i> I	<i>Pml</i> I	<i>SgrA</i> I	<i>Tth111</i> I
<i>Age</i> I	<i>Bst1107</i> I	<i>Nde</i> I	<i>Ppu10</i> I	<i>SnaB</i> I	<i>Xcm</i> I
<i>Asc</i> I	<i>Bst98</i> I	<i>Nru</i> I	<i>PpuM</i> I	<i>Spe</i> I	
<i>Avr</i> II	<i>BstE</i> II	<i>Nsi</i> I	<i>Psp5</i> II	<i>Sph</i> I	
<i>BbrP</i> I	<i>Csp</i> I	<i>Pac</i> I	<i>Rsr</i> II	<i>Spl</i> I	

Table 4. Restriction Enzymes That Cut the pGL3(R2.1)-Basic Vector 6 or More Times.

<i>Acc</i> I	<i>Bsr</i> I	<i>Dsa</i> I	<i>Hph</i> I	<i>Msp</i> I	<i>ScrF</i> I
<i>Acy</i> I	<i>Bsr S</i> I	<i>Eae</i> I	<i>Hsp92</i> I	<i>Nae</i> I	<i>SfaN</i> I
<i>Alu</i> I	<i>Bst71</i> I	<i>Fnu4H</i> I	<i>Hsp92</i> II	<i>Nde</i> II	<i>Taq</i> I
<i>AspH</i> I	<i>BstO</i> I	<i>Fok</i> I	<i>Mae</i> I	<i>NgoM IV</i>	<i>Tru9</i> I
<i>Ban</i> I	<i>BstU</i> I	<i>Hae</i> II	<i>Mae</i> II	<i>Nla</i> III	<i>Xho</i> II
<i>Bbv</i> I	<i>Cfo</i> I	<i>Hae</i> III	<i>Mae</i> III	<i>Nla</i> IV	
<i>BsaO</i> I	<i>Cfr10</i> I	<i>Hga</i> I	<i>Mbo</i> I	<i>Ple</i> I	
<i>BsaH</i> I	<i>Dde</i> I	<i>Hha</i> I	<i>Mbo</i> II	<i>Rsa</i> I	
<i>BsaJ</i> I	<i>Dpn</i> I	<i>Hinf</i> I	<i>Mnl</i> I	<i>Sau3A</i> I	
<i>Bsp1286</i> I	<i>Dpn</i> II	<i>Hpa</i> II	<i>Mse</i> I	<i>Sau96</i> I	

Note: The enzymes listed in boldface type are available from Promega.

B. pGL3(R2.2)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3'-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487822) and online at: www.promega.com/vectors/

Table 5. Restriction Enzymes That Cut the pGL3(R2.2)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Acc I	2	688, 2214	Eco81 I	2	701, 1301
Acc65 I	1	1	EcoICR I	1	9
Afl II	1	1767	EcoN I	3	277, 700, 1705
Afl III	2	15, 2463	EcoR I	1	1737
Alw26 I	4	1111, 1409, 3417, 4193	Ehe I	3	369, 1035, 1167
Alw44 I	2	2777, 4023	Fse I	2	1499, 1964
AlwN I	1	2879	Fsp I	3	910, 3578, 4751
Apa I	1	118	Hinc II	2	2105, 2215
Ava I	3	26, 32, 1810	Hind II	2	2105, 2215
Ava II	4	1209, 1392, 3494, 3716	Hind III	1	53
Bal I	2	1069, 1594	Hpa I	1	2105
BamH I	1	2207	Kas I	3	367, 1033, 1165
Ban II	4	11, 33, 118, 4434	Kpn I	1	5
Bbe I	3	371, 1037, 1169	Mlu I	1	15
Bbs I	1	2292	MspA1 I	5	151, 162, 2805, 3050, 3991
Bcl I	3	668, 1409, 1703	Nar I	3	368, 1034, 1166
Bgl I	5	88, 1492, 1667, 3476, 4744	Nci I	5	27, 28, 2843, 3539, 3890
Bgl II	1	36	Nco I	1	86
Bsa I	1	3417	Nhe I	1	21
BsaA I	2	1290, 4505	Not I	1	4854
BsaB I	3	535, 1702, 2206	Nsp I	3	579, 834, 2467
BsaM I	3	60, 2026, 2119	PaeR7 I	1	32
Bsm I	3	60, 2026, 2119	PshA I	1	2278
Bsp120 I	1	114	PspA I	1	26
BspH I	3	671, 3183, 4191	Pst I	3	347, 488, 1466
BspM I	2	1019, 4984	Pvu I	2	3726, 4772
BsrG I	1	578	Pvu II	1	162
BssS I	3	1015, 2636, 4020	Sac I	1	11
Bst98 I	1	1767	Sal I	1	2213
BstX I	1	1206	Sca I	3	253, 3836, 4919
BstZ I	3	1958, 1962, 4854	Sin I	4	1209, 1392, 3494, 3716
Bsu36 I	2	701, 1301	Sma I	1	28
Cla I	3	2200, 4912, 5016	Srf I	1	28
Dra I	4	2166, 3222, 3241, 3933	Ssp I	3	4160, 4713, 4828
Dra II	4	114, 115, 480, 1659	Sty I	3	86, 1199, 1655
Dra III	2	1779, 4508	Tfi I	2	2438, 4909
Drd I	2	2571, 4552	Vsp I	1	3528
Eag I	3	1958, 1962, 4854	Xba I	1	1945
Ear I	5	172, 283, 2347, 4151, 4789	Xho I	1	32
EclHK I	1	3356	Xma I	1	26
Eco47 III	2	1075, 2339	Xmn I	2	1756, 3955
Eco52 I	3	1958, 1962, 4854			

Note: The enzymes listed in boldface type are available from Promega.



Table 6. Restriction Enzymes That Do Not Cut the pGL3(R2.2)-Basic Vector.

<i>Aat</i> II	<i>Blp</i> I	<i>EcoR</i> V	<i>Pme</i> I	<i>Sgf</i> I	<i>Swa</i> I
<i>AccB7</i> I	<i>Bpu</i> 1102 I	<i>I-Ppo</i> I	<i>Pml</i> I	<i>SgrA</i> I	<i>Tth</i> 111 I
<i>Acc</i> III	<i>BssH</i> II	<i>Nde</i> I	<i>Ppu</i> 10 I	<i>SnaB</i> I	<i>Xcm</i> I
<i>Age</i> I	<i>Bst</i> 1107 I	<i>Nru</i> I	<i>PpuM</i> I	<i>Spe</i> I	
<i>Asc</i> I	<i>BstE</i> II	<i>Nsi</i> I	<i>Psp5</i> II	<i>Sph</i> I	
<i>Avr</i> II	<i>Csp</i> I	<i>Pac</i> I	<i>Rsr</i> II	<i>Spl</i> I	
<i>BbrP</i> I	<i>Csp45</i> I	<i>PflM</i> I	<i>Sac</i> II	<i>Sse8387</i> I	
<i>Bbu</i> I	<i>Eco72</i> I	<i>PinA</i> I	<i>Sfi</i> I	<i>Stu</i> I	

Table 7. Restriction Enzymes That Cut the pGL3(R2.2)-Basic Vector 6 or More Times.

<i>Aci</i> I	<i>Bsr</i> I	<i>Dsa</i> I	<i>Hph</i> I	<i>Msp</i> I	<i>ScrF</i> I
<i>Acy</i> I	<i>BsrS</i> I	<i>Eae</i> I	<i>Hsp92</i> I	<i>Nae</i> I	<i>SfaN</i> I
<i>Alu</i> I	<i>Bst71</i> I	<i>Fnu4H</i> I	<i>Hsp92</i> II	<i>Nde</i> II	<i>Taq</i> I
<i>AspH</i> I	<i>BstO</i> I	<i>Fok</i> I	<i>Mae</i> I	<i>NgoM</i> IV	<i>Tru9</i> I
<i>Ban</i> I	<i>BstU</i> I	<i>Hae</i> II	<i>Mae</i> II	<i>Nla</i> III	<i>Xho</i> II
<i>Bbv</i> I	<i>Cfo</i> I	<i>Hae</i> III	<i>Mae</i> III	<i>Nla</i> IV	
<i>BsaO</i> I	<i>Cfr10</i> I	<i>Hga</i> I	<i>Mbo</i> I	<i>Ple</i> I	
<i>BsaH</i> I	<i>Dde</i> I	<i>Hha</i> I	<i>Mbo</i> II	<i>Rsa</i> I	
<i>BsaJ</i> I	<i>Dpn</i> I	<i>Hint</i> I	<i>Mnl</i> I	<i>Sau3A</i> I	
<i>Bsp1286</i> I	<i>Dpn</i> II	<i>Hpa</i> II	<i>Mse</i> I	<i>Sau96</i> I	

Note: The enzymes listed in boldface type are available from Promega.

C. pHG(R2.1)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3'-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487823) and online at: www.promega.com/vectors/

Table 8. Restriction Enzymes That Cut the pHG(R2.1)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Aat II	1	785	EcoR V	1	573
Acc I	1	1425	Fok I	4	295, 2533, 2714, 3001
Acc65 I	1	1	Fse I	1	1175
Acy I	2	782, 3104	Fsp I	2	2789, 3962
Afl III	2	15, 1674	Hae II	5	1130, 1552, 1922, 3561, 3569
Alw26 I	3	628, 2628, 3404	Hga I	5	964, 1785, 2363, 3093, 3494
Alw44 I	2	1988, 3234	Hinc II	2	1316, 1426
AlwN I	1	2090	Hind II	2	1316, 1426
AspH I	5	11, 485, 1992, 3153, 3238	Hind III	1	53
Ava I	4	26, 32, 109, 1039	Hpa I	1	1316
Ava II	2	2705, 2927	Hsp92 I	2	782, 3104
BamH I	1	1418	Kpn I	1	5
Ban II	5	11, 33, 153, 293, 3645	Mlu I	1	15
Bbs I	1	1503	MspA1 I	3	2016, 2261, 3202
Bcl I	2	128, 581	Nae I	4	1062, 1173, 1544, 3613
Bgl I	3	88, 2687, 3955	Nci I	5	27, 28, 2054, 2750, 3101
Bgl II	1	36	Nco I	1	86
Bsa I	2	628, 2628	NgoM IV	4	1060, 1171, 1542, 3611
BsaA I	1	3716	Nhe I	1	21
BsaB I	2	847, 1417	Not I	1	4065
BsaH I	2	782, 3104	Nru I	1	749
BsaM I	3	60, 1237, 1330	Nsi I	1	307
Bsm I	3	60, 1237, 1330	Nsp I	1	1678
BspH I	2	2394, 3402	PaeR7 I	1	32
BspM I	1	4195	Ppu10 I	1	303
BssS I	2	1847, 3231	PshA I	1	1489
BstZ I	3	1169, 1173, 4065	PspA I	1	26
Bsu36 I	1	674	Pvu I	2	2937, 3983
Cfr10 I	5	1060, 1171, 1542, 2647, 3611	Sac I	1	11
Cla I	3	1411, 4123, 4227	Sal I	1	1424
Dra I	4	1377, 2433, 2452, 3144	Sca I	2	3047, 4130
Dra II	1	933	Sin I	2	2705, 2927
Dra III	2	276, 3719	Sma I	1	28
Drd I	3	1159, 1782, 3763	Srf I	1	28
Dsa I	2	86, 293	Ssp I	3	3371, 3924, 4039
Eae I	4	1169, 1173, 2955, 4065	Sty I	2	86, 95
Eag I	3	1169, 1173, 4065	Tfi I	3	199, 1649, 4120
Ear I	4	587, 1558, 3362, 4000	Tth111 I	1	784
EclHK I	1	2567	Vsp I	1	2739
Eco47 III	1	1550	Xba I	1	1156
Eco52 I	3	1169, 1173, 4065	Xho I	1	32
Eco81 I	1	674	Xma I	1	26
EcoCR I	1	9	Xmn I	2	622, 3166
EcoR I	1	1020			

Note: The enzymes listed in boldface type are available from Promega.



Table 9. Restriction Enzymes That Do Not Cut the phRG(R2.1)-Basic Vector.

<i>Acc</i> B7 I	<i>Bbr</i> P I	<i>Bst</i> E II	<i>Nar</i> I	<i>Pst</i> I	<i>Sph</i> I
<i>Acc</i> III	<i>Bbu</i> I	<i>Bst</i> X I	<i>Nde</i> I	<i>Pvu</i> II	<i>Spl</i> I
<i>Afl</i> II	<i>Blp</i> I	<i>Csp</i> I	<i>Pac</i> I	<i>Rsr</i> II	<i>Sse</i> 8387 I
<i>Age</i> I	<i>Bpu</i> 1102 I	<i>Csp</i> 45 I	<i>Pfl</i> M I	<i>Sac</i> II	<i>Stu</i> I
<i>Apa</i> I	<i>Bsp</i> 120 I	<i>Eco</i> 72 I	<i>Pin</i> A I	<i>Sfi</i> I	<i>Swa</i> I
<i>Asc</i> I	<i>Bsr</i> G I	<i>Eco</i> N I	<i>Pme</i> I	<i>Sgf</i> I	<i>Xcm</i> I
<i>Avr</i> II	<i>Bss</i> H II	<i>Ehe</i> I	<i>Pml</i> I	<i>Sgr</i> A I	
<i>Bal</i> I	<i>Bst</i> 1107 I	<i>I-Ppo</i> I	<i>Ppu</i> M I	<i>Sna</i> B I	
<i>Bbe</i> I	<i>Bst</i> 98 I	<i>Kas</i> I	<i>Psp</i> 5 II	<i>Spe</i> I	

Table 10. Restriction Enzymes That Cut the phRG(R2.1)-Basic Vector 6 or More Times.

<i>Aci</i> I	<i>Bsr</i> S I	<i>Fnu</i> 4H I	<i>Mae</i> II	<i>Nla</i> III	<i>Taq</i> I
<i>Alu</i> I	<i>Bst</i> 71 I	<i>Hae</i> III	<i>Mae</i> III	<i>Nla</i> IV	<i>Tru</i> 9 I
<i>Ban</i> I	<i>Bst</i> O I	<i>Hha</i> I	<i>Mbo</i> I	<i>Ple</i> I	<i>Xho</i> II
<i>Bbv</i> I	<i>Bst</i> U I	<i>Hint</i> I	<i>Mbo</i> II	<i>Rsa</i> I	
<i>Bsa</i> O I	<i>Cfo</i> I	<i>Hpa</i> II	<i>Mnl</i> I	<i>Sau</i> 3A I	
<i>Bsa</i> J I	<i>Dde</i> I	<i>Hph</i> I	<i>Mse</i> I	<i>Sau</i> 96 I	
<i>Bsp</i> 1286 I	<i>Dpn</i> I	<i>Hsp</i> 92 II	<i>Msp</i> I	<i>Scr</i> F I	
<i>Bsr</i> I	<i>Dpn</i> II	<i>Mae</i> I	<i>Nde</i> II	<i>Sfa</i> N I	

Note: The enzymes listed in boldface type are available from Promega.



D. pHG(R2.2)-Basic Vector Restriction Enzyme Tables

The following restriction enzyme tables were constructed using DNASTAR® sequence analysis software. Please note that we have not verified this information by restriction digestion with each enzyme listed. The location given specifies the 3'-end of the cut DNA (the base to the left of the cut site). For more information on the cut sites of these enzymes, or if you identify a discrepancy, please contact your local Promega Branch or Distributor. In the U.S., contact Promega Technical Services at 800-356-9526. Vector sequences are also available in the GenBank® database (GenBank®/EMBL Accession Number AY487824) and online at: www.promega.com/vectors/

Table 11. Restriction Enzymes That Cut the pHG(R2.2)-Basic Vector Between 1 and 5 Times.

Enzyme	# of Sites	Location	Enzyme	# of Sites	Location
Aat II	1	785	EcoR I	1	1020
Acc I	1	1497	EcoR V	1	573
Acc65 I	1	1	Fok I	4	295, 2605, 2786, 3073
Acy I	2	782, 3176	Fse I	1	1247
Afl II	1	1050	Fsp I	2	2861, 4034
Afl III	2	15, 1746	Hae II	5	1184, 1624, 1994, 3633, 3641
Alw26 I	3	628, 2700, 3476	Hga I	5	964, 1857, 2435, 3165, 3566
Alw44 I	2	2060, 3306	Hinc II	2	1388, 1498
AlwN I	1	2162	Hind II	2	1388, 1498
AspH I	5	11, 485, 2064, 3225, 3310	Hind III	1	53
Ava I	4	26, 32, 109, 1093	Hpa I	1	1388
Ava II	2	2777, 2999	Hsp92 I	2	782, 3176
BamH I	1	1490	Kpn I	1	5
Ban II	5	11, 33, 153, 293, 3717	Mlu I	1	15
Bbs I	1	1575	MspA1 I	3	2088, 2333, 3274
Bcl I	2	128, 581	Nae I	4	1116, 1245, 1616, 3685
Bgl I	3	88, 2759, 4027	Nci I	5	27, 28, 2126, 2822, 3173
Bgl II	1	36	Nco I	1	86
Bsa I	2	628, 2700	NgoM IV	4	1114, 1243, 1614, 3683
BsaA I	1	3788	Nhe I	1	21
BsaB I	2	847, 1489	Not I	1	4137
BsaH I	2	782, 3176	Nru I	1	749
BsaM I	3	60, 1309, 1402	Nsi I	1	307
Bsm I	3	60, 1309, 1402	Nsp I	1	1750
BspH I	2	2466, 3474	PaeR7 I	1	32
BspM I	1	4267	Ppu10 I	1	303
BssS I	2	1919, 3303	PshA I	1	1561
Bst98 I	1	1050	PspA I	1	26
BstZ I	3	1241, 1245, 4137	Pvu I	2	3009, 4055
Bsu36 I	1	674	Sac I	1	11
Cfr10 I	5	1114, 1243, 1614, 2719, 3683	Sal I	1	1496
Cla I	3	1483, 4195, 4299	Sca I	2	3119, 4202
Dra I	4	1449, 2505, 2524, 3216	Sin I	2	2777, 2999
Dra II	1	933	Sma I	1	28
Dra III	3	276, 1062, 3791	Srf I	1	28
Drd I	2	1854, 3835	Ssp I	3	3443, 3996, 4111
Dsa I	3	86, 293, 1080	Sty I	2	86, 95
Eae I	4	1241, 1245, 3027, 4137	Tfi I	3	199, 1721, 4192
Eag I	3	1241, 1245, 4137	Tth111 I	1	784
Ear I	4	587, 1630, 3434, 4072	Vsp I	1	2811
EclHK I	1	2639	Xba I	1	1228
Eco47 III	1	1622	Xho I	1	32
Eco52 I	3	1241, 1245, 4137	Xma I	1	26
Eco81 I	1	674	Xmn I	3	622, 1039, 3238
EcoICR I	1	9			



Table 12. Restriction Enzymes That Do Not Cut the phRG(R2.2)-Basic Vector.

AccB7 I	Bbu I	Csp I	Pac I	Rsr II	Sse8387 I
Acc III	Blp I	Csp45 I	PflM I	Sac II	Stu I
Age I	Bpu1102 I	Eco72 I	PinA I	Sfi I	Swa I
Apa I	Bsp120 I	EcoN I	Pme I	Sgf I	Xcm I
Asc I	BsrG I	Ehe I	Pml I	SgrA I	
Avr II	BssH II	I-Ppo I	PpuM I	SnaB I	
Bal I	Bst1107 I	Kas I	Psp5 II	Spe I	
Bbe I	BstE II	Nar I	Pst I	Sph I	
BbrP I	BstX I	Nde I	Pvu II	Spl I	

Table 13. Restriction Enzymes That Cut the phRG(R2.2)-Basic Vector 6 or More Times.

Aci I	BsrS I	Fnu4H I	Mae II	Nla III	Taq I
Alu I	Bst71 I	Hae III	Mae III	Nla IV	Tru9 I
Ban I	BstO I	Hha I	Mbo I	Ple I	Xho II
Bbv I	BstU I	Hinf I	Mbo II	Rsa I	
BsaO I	Cfo I	Hpa II	Mnl I	Sau3A I	
BsaJ I	Dde I	Hph I	Mse I	Sau96 I	
Bsp1286 I	Dpn I	Hsp92 II	Msp I	ScrF I	
Bsr I	Dpn II	Mae I	Nde II	SfaN I	

Note: The enzymes listed in boldface type are available from Promega.

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(g) U.S. Pat. No. 5,670,356 has been issued to Promega Corporation for a modified luciferase technology.

(h) U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

(i) U.S. Pat. No. 5,744,320, Australian Pat. No. 721172 and European Pat. No. 0 833 939 have been issued to Promega Corporation for quenching reagents and assays for enzyme-mediated luminescence. Other patents are pending.

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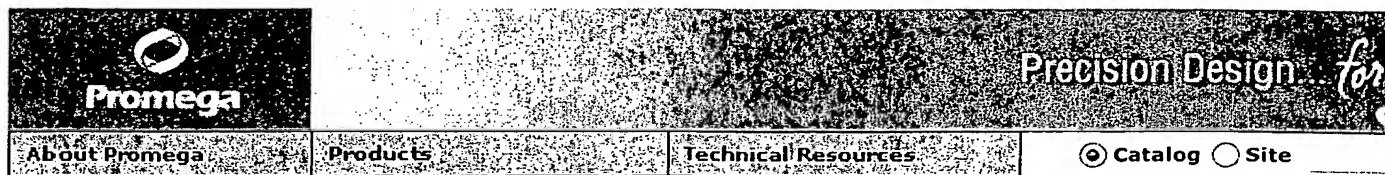
(l) U.S. Pat. No. 5,391,487 has been issued to Promega Corporation for Restriction Endonuclease *Sgf I*.

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Product	Introduced	Protocol
Pronto!™ Plus Systems	Oct-2003	TM243
Pronto!™ Plus System 1		
Pronto!™ Plus System 2		
Pronto!™ Plus System 3		
Pronto!™ Plus System 4		
Pronto!™ Plus System 5		
Pronto!™ Plus System 6		
MagneSil® Total RNA Mini-Isolation System	Oct-2003	TB328
MagneSil® KF, Genomic System	Jun-2003	TB322
MagneSil® KF, Paramagnetic Particles, 40ml		
Lysis Buffer, KF, 160ml		
Wizard® SV 96 PCR Clean-Up System, 100 x 96 preps	May-2003	TB311
MagneSil® Genomic, Fixed Tissue System	May-2003	TB319
PolyATtract® Automated System, 4 x 96 preps	Apr-2003	TB321
RNasin® Plus RNase Inhibitor, 2,500u	Apr-2003	
10,000u		
siLentGene™ U6 Cassette RNA Interference System	Mar-2003	TM061

PROTEOMICS

Product	Introduced	Protocol
U6 Hairpin Cloning Systems	Dec-2003	TM246
siSTRIKE™ U6 Hairpin Cloning System—Basic		
siSTRIKE™ U6 Hairpin Cloning System—Puromycin		
siSTRIKE™ U6 Hairpin Cloning System—Hygromycin		
siSTRIKE™ U6 Hairpin Cloning System—Neomycin		
MagneGST™ Pull-Down System	Dec-2003	TM249
HisLink™ Protein Purification Resin	Dec-2003	TB327
Rapid Response™ Reporter Vectors	Dec-2003	TM242
pGL3(R2.1)-Basic Vector, 20µg		
pGL3(R2.2)-Basic Vector, 20µg		

pRG(R2.1)-Basic Vector, 20µg		
pRG(R2.2)-Basic Vector, 20µg		
FastBreak™ Cell Lysis Reagent	Dec-2003	PIV857
10ml		
40ml		
100ml		
EnduRen™ Live Cell Substrate	Dec-2003	TM244
0.34mg		
3.4mg		
34mg		
MagneGST™ Protein Purification System	Oct-2003	TM240
40 reactions		
200 reactions		
MagneGST™ Glutathione Particles	Oct-2003	TM240
4ml		
20ml		
siFECT™ siRNA Transfection Reagent	Oct-2003	TB326
0.1ml		
0.4ml		
1.0ml		
Chroma-Glo™ Luciferase Assay System,	Jul-2003	TM062
10ml		
100ml		
10 x 100ml		
pCBR-Basic Vector		
pCBR-Control Vector		
pCBG68-Basic Vector		
pCBG-Control Vector		
pCBG99-Basic Vector		
pCBG99-Control Vector		
T7 RiboMAX™ Express RNAi System	Apr-2003	TB316
siLentGene™ U6 Cassette RNA	Mar-2003	TM061
Interference System		
Beta-Glo™ Assay System,	Feb-2003	TM239
10ml		
100ml		
10 x 100ml		
Monster Green™ Fluorescent Protein	Feb-2003	TB320
phMGFP Vector, 20µg		

CELLULAR ANALYSIS

Product	Introduced	Protocol
ProFluor™ Tyrosine Phosphatase Assay	Jan-2004	TB334
4 plate		
8 plate		
Caspase-Glo™ 8 Assay,	Dec-2003	TB332
2.5ml		
10ml		
100ml		
Caspase-Glo™ 9 Assay,	Dec-2003	TB333
2.5ml		
10ml		
100ml		

ProFluor™ Src-Family Kinase Assay, 4 plate 8 plate	Dec-2003	TB331
p450-Glo™ CYP450 Assays	Jul-2003	TB325
p450-Glo™ CYP1A1 Assay p450-Glo™ CYP1A1 Assay		
p450-Glo™ CYP1B1 Assay p450-Glo™ CYP1B1 Assay		
p450-Glo™ CYP1A2 Assay p450-Glo™ CYP1A2 Assay		
p450-Glo™ CYP2C8 Assay p450-Glo™ CYP2C8 Assay		
p450-Glo™ CYP2C9 Assay p450-Glo™ CYP2C9 Assay		
p450-Glo™ CYP3A4 Assay p450-Glo™ CYP3A4 Assay		
p450-Glo™ CYP3A7 Assay p450-Glo™ CYP3A7 Assay		
CytoTox-ONE™ Homogeneous Membrane Integrity Assay, HTP	Jul-2003	TB306
Also available: CytoTox-ONE™ Homogeneous Membrane Integrity Assay, 200-800 assays 1,000-4,000 assays		
ProFluor™ Ser/Thr Phosphatase Assay, 4 plate 8 plate	Jul-2003	TB324
Caspase-Glo™ 3/7 Assay, 2.5ml 10ml 100ml	Jun-2003	TB323
CellTiter-Blue™ Cell Viability Assay, 20ml 100ml	Jan-2003	TB317

GENETIC IDENTITY

Product	Introduced	Protocol
Y Chromosome Deletion Detection System, Version 2.0	Oct-2003	TM248
PowerPlex® Y System, 200 reactions 50 reactions	Aug-2003	TMD018

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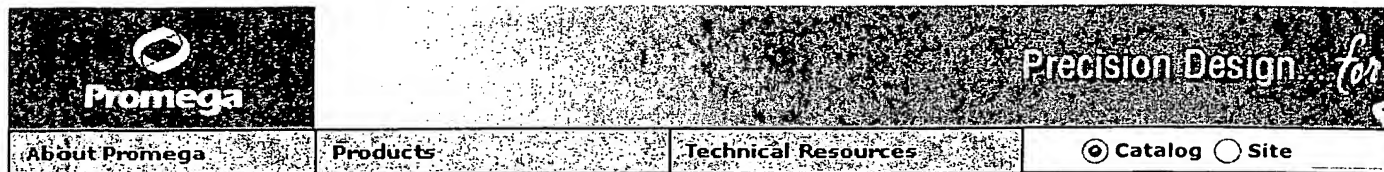
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pGL3(R2.2)-Basic Vector	E6441	20µg	Each	\$ 244.00	1
phRG(R2.1)-Basic Vector	E6451	20µg	Each	\$ 244.00	1
phRG(R2.2)-Basic Vector	E6461	20µg	Each	\$ 244.00	1

[Component Listing](#)**Description**

The Rapid Response™ Reporter Vectors^(a,b,c) set a new standard for genes in their ability to respond more quickly and sensitively to transcription. The Rapid Response™ Reporters consist of fast- and responding luciferase genes that generate a greater magnitude of in less time, enabling you to significantly decrease incubation time and quantitate smaller relative changes in gene expression. Available in synthetic firefly or two synthetic *Renilla* luciferase vector configurations. Rapid Response™ Reporters are designed for use with Promega's standard luminescence assay reagents to obtain the most rapid and accurate reporter gene measurements.

Two configurations of the Rapid Response™ Reporters are available to select the optimal reporter for your application requirements. In the presence of mRNA and protein degradation sequences, the pGL3(R2.1)-Basic^(a,b,c,d) and phRG(R2.2)-Basic^(a,b,c,e) Vectors provide the fastest and greatest magnitude of response for firefly and *Renilla* luciferases, respectively. The pGL3(R2.1)-Basic^(a,b,c,d) and phRG(R2.1)-Basic^(a,b,c,d) Vectors, which contain protein degradation sequences, also achieve faster response rates and high fold induction, yet they retain higher total expression. All Rapid Response™ Reporters are provided in a Basic configuration that contains a multiple cloning site to allow for maximum flexibility in cloning a regulatory element of interest.

Features

- **Up to Fourfold Faster Reporter Response:** Decrease incubation time by up to 75% to reduce the risk of secondary effects and improve screening efficiency.
- **Greater Sensitivity:** Achieve a greater magnitude of reporter response and quantitate smaller relative changes in gene expression.
- **Synthetic Gene Design:** Minimize the risk of nonspecific activation with reporters that are free of most known consensus sequence transcription factor binding sites. Achieve high expression levels with codons optimized for mammalian systems.
- **Compatible with Promega's Industry Standard Detection Reagents:** Enjoy rapid, robust and easy-to-use assays in standard dual-reporter formats.

Protocol**Vector Sequence**[Technical Manual #TM242.](#)[pGL3\(R2.1\)-Basic Vector sequence.](#)[pGL3\(R2.2\)-Basic Vector sequence.](#)[phRG\(R2.1\)-Basic Vector sequence.](#)[phRG\(R2.2\)-Basic Vector sequence.](#)**Storage Conditions**

Store at -20°C.

GenBank®/EMBL**Accession Number**

pGL3(R2.1)-Basic, AY487821; pGL3(R2.2)-Basic, AY487822; phRG(R2.1)-Basic, AY487823; phRG(R2.2)-Basic, AY487824.

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PATENT COOPERATION TREATY
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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 2510548/VPA/sjw	<div style="display: flex; justify-content: space-between;"> <div style="text-align: center;">FOR FURTHER ACTION</div> <div style="text-align: right; font-size: small;">see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</div> </div>	
International application No. PCT/AU02/00351	International filing date (day/month/year) 8 March 2002	(Earliest) Priority Date (day/month/year) 9 March 2001
Applicant GENE STREAM PTY LTD et al		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

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☒ filed together with the international application in computer readable form.

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☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

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2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title, ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract, ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure

☐ because this figure better characterizes the invention

☒ None of the figures

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU02/00351

A. CLASSIFICATION OF SUBJECT MATTERInt. Cl. ⁷: C12N 15/67

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

SEE BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SEE BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (WPIDS): untranslated region and (destab? or stab?)

DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	WO 99/14346, A (Sequitur, Inc) 25.03.99; see pages 4 and 11-25.	1-10; 22 and 26
X,Y	WO 95/29244, A (Wisconsin Alumni Research Foundation) 02.11.95; see page 5 and claims	1-10, 22 and 26
X,Y	Oncogene 11, pp 2127-2134 (1995) Veyrune et al "c-fos mRNA instability determinants present within both the coding and the 3' non coding region link the degradation of this mRNA to its translation".	1-10, 22 and 26

☒ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
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"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 May 2002

Date of mailing of the international search report

17 MAY 2002

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE
 PO BOX 200, WODEN ACT 2606, AUSTRALIA
 E-mail address: pct@ipaaustralia.gov.au
 Facsimile No. (02) 6285 3929

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MADHU K. JOGIA

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	Nucleic Acids Research 27, pp 1164-1673 (1999) Maurer et al "An AU-rich sequence in the 3'-UTR of plasminogen activator inhibitor type 2 (PAI-2) mRNA promotes PAI-2 mRNA decay and provides a binding site for nuclear HuR"	1-10, 22 and 26
X, Y	Molecular and Cellular Biology 21, pp 721-730 (Feb 2001) Dean et al "The 3' Untranslated region of tumor necrosis factor alpha mRNA is a target of the mRNA-stabilizing factor HuR".	1-10, 22 and 26
X	J Biol Chem 275, pp 30248-30255 (2000) Provost et al "Length increase of the human α -globin 3'-untranslated region disrupts stability of the pre-mRNA but not that of the mature mRNA"	1-10, 22 and 26
X	J Biol Chem 275, pp 12963-12969 (2000) Short et al "Structural determinants for post-transcriptional stabilization of lactate dehydrogenase A mRNA by the protein kinase C signal pathway"	1-10, 22 and 26
X	J Biol Chem 273, pp 15749-15757 (1998) Yeilding et al "c-myc mRNA is down-regulated during myogenic differentiation by accelerated decay that depends on translation of regulatory coding elements".	1-10, 22 and 26
X	Molecular and Cellular Biology 17, pp 2698-2707 (1997) Yeilding et al "Coding elements in exons 2 and 3 target c-myc mRNA downregulation during myogenic differentiation".	1-10, 22 and 26

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Information on patent family members

International application No.

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Patent Document Cited in Search Report				Patent Family Member			
WO	9914346	AU	93193/98	CA	2304982	EP	1021549
WO	9529244	AU	22959/95	US	5587300		
END OF ANNEX							